

DISTRIBUTION OF NONPROTEIN IMINO AND SULPHUR AMINO ACIDS IN *ZAPOTECA*¹

JOHN T. ROMEO²

ABSTRACT

Seeds of eight and leaves of nine species of *Zapoteca* were surveyed for nonprotein sulphur-containing amino acids and nonprotein imino acids, and the patterns compared with those of *Calliandra* species. S-(β -carboxyethyl)-cysteine, the major free seed amino acid in all *Calliandra* species examined, and S-(β -carboxyisopropyl)-cysteine, a lesser constituent, were not detected in *Zapoteca* species. Conversely, an uncharacterized compound not significantly present in *Calliandra* is the major free amino acid of *Zapoteca*. The major imino acids of *Zapoteca*, trans-trans-4,5-dihydroxypipelicolic acid, trans-4-hydroxypipelicolic acid, and cis-5-hydroxypipelicolic acid, are a consistent feature of the genus, but these same compounds are also found together in many *Calliandra* species. The chemical data are interpreted as supportive evidence for the distinctiveness of these two closely allied groups of plants.

Calliandra (Mimosoideae) is a large heterogeneous group of tropical-subtropical woody shrubs and trees found mainly in the Americas, extending from the southern U.S. to Argentina. About 200 species have been described. *Zapoteca*, a new genus (Hernández, 1986) consists of a morphologically homogeneous group of 25 taxa distributed largely in southern Mexico. The group includes all plants placed previously under Bentham's series *Laetevirentes* of *Calliandra* (1875).

Species of both genera are characterized by large amounts of rare unusual imino acids that accumulate in both seeds and leaves. Nine compounds, derivatives of pipelicolic acid, previously have been isolated from various *Calliandra* species (Marlier et al., 1972, 1979; Bleeker & Romeo, 1981, 1983; Romeo et al., 1983). They include four monohydroxylated pipelicolic acids (trans-4-, trans-5-, cis-4-, and cis-5-hydroxypipelicolic acids); four dihydroxylated pipelicolic acids (trans-cis-, trans-trans-, cis-trans-, and cis-cis-4,5-dihydroxypipelicolic acids); and trans-4-acetylaminopipelicolic acid. The last two compounds are known only from *Calliandra*. Populational sampling of a number of species has established that there is essentially no intraspecific variability in the rare compounds (Romeo, 1984). Complementary laboratory experiments have shown that the compounds are stable under various conditions of imposed water and UV stress (Bleeker & Romeo, in prep.; Balis & Romeo, unpubl. data). The rarity of the compounds coupled to their apparent ecological stability indi-

cates that they are suitable for use in taxonomic studies. Analysis of *Calliandra* leaf material of over 100 species has produced several different chemical patterns (Romeo, 1984 and unpubl. data). The usefulness of these chemical groups in helping to establish a natural grouping of species within the large genus *Calliandra*, however, is yet to be determined. *Calliandra* is currently undergoing systematic revision, and the chemical findings will be interpreted in conjunction with results emerging from other fronts (see below).

The seeds of *Calliandra* are characterized additionally by large amounts of nonprotein sulphur-containing amino acids. S-(β -carboxyethyl)-cysteine (S-CEC) is the major compound. It is rapidly metabolized upon germination and is absent from mature foliage, but continues to appear in new emerging leaves up to 70 days after germination (Swain & Romeo, 1986). Lesser amounts of other related S-containing amino acids are also present in seeds. S-(β -carboxyisopropyl)-cysteine (S-CIC), djenkolic acid, and acetyldjenkolic acid are found frequently along with uncharacterized oxidized derivatives of these compounds (Krauss & Reinbothe, 1970; Romeo & Swain, unpubl.).

The presence of these two classes of unusual amino acids in *Calliandra* has led to a large-scale study of the distribution of these compounds in leaves and seeds. *Calliandra*, which has received little significant taxonomic treatment since the monograph by Bentham (1875), is now the focus

¹ This material is based upon work supported by the National Science Foundation under grant no. BSR 8400277. I thank Lee Swain, who played a major role in the collection of chemical data, for his assistance.

² Department of Biology, University of South Florida, Tampa, Florida 33620.

of much research (Forero, 1984). In addition to morphological studies, which have been initiated by Forero (Colombian species), Ataíde (Brazilian species), and Hernández (Central American-Mexican species), developmental, palynological, chromosomal, and chemical studies are underway. The work described herein is part of this multidisciplinary project. With the establishment of a new genus *Zapoteca* (Hernández, 1986) to include several species previously considered under *Calliandra*, it is an appropriate time to put forth some of the data that are emerging from the chemical studies. The purpose of this paper is to present chemical data on *Zapoteca* species, and to discuss the significance of these findings particularly in relation to *Calliandra*.

MATERIALS AND METHODS

Collection of Material. Species are listed with voucher data in Tables 1 and 2.

Extraction and chromatographic methods. One hundred mg of material were ground and extracted by the method described in Romeo et al. (1983). A MeOH-CHCl₃-H₂O (12:5:1) extract was separated into an upper aqueous and lower CHCl₃ layer by addition of H₂O and CHCl₃. The aqueous phase was removed, evaporated to dryness and redissolved in 25% EtOH. This was used for paper chromatography and high voltage paper electrophoresis. Samples for quantitative analysis on the amino acid analyzer (Dionex D-300) were prepared by evaporating aliquots of the above solution and redissolving them in 0.2 M Na⁺ buffer (Dionex Femto buffer 1A, pH 2.0). Chromatographic solvents used were 1) BuOH-HOAc-H₂O (12:3:5), 2) 80% PhOH-H₂O (w/v) in the presence of NH₃ vapor, 3) BuOH-HCO₂H-H₂O (15:3:2). High voltage electrophoresis was performed on paper in buffer (pH 1.9).

Identification of compounds. Compounds were identified from R_f values and ionic mobilities (Seneviratne & Fowden, 1968; Romeo et al., 1983), comparison with authentic standards, and color reactions with the location reagents ninhydrin, isatin, and iodoplatinate.

RESULTS

There is a striking dichotomy in sulphur chemistry between *Calliandra* as circumscribed here, which includes members of Bentham's series *Macrophyllae*, *Nitidae*, and *Racemosae* (seed material of the small series *Pedicellatae* is not yet available for study), and the new genus *Za-*

TABLE 1. Distribution of sulphur-containing amino acids in seeds of species of *Zapoteca* compared with *Calliandra*. S-CEC = S-(β-carboxyethyl)-cysteine; S-CIC = S-(β-carboxyisopropyl)-cysteine; S-2 = uncharacterized amino acid.

Bentham Series	No. Species* Examined	Compounds		
		S-CEC	S-CIC	S-2
Laetevirentes (<i>Zapoteca</i>)	8	—	—	++
Macrophyllae	8	+++	+	T
Nitidae	23	+++	+	T
Racemosae	8	+++	+	T

+++ = > 10 mg/g dry wt; ++ = 1–10 mg/g dry wt; + = 0.1–1 mg/g dry wt; T = <0.1 mg/g dry wt; — = not detected.

* Species are listed by name, collection number, and herbarium where voucher is on file. Collectors: D = Delgado, DN = Neill, DS = Stevens, F = Forero, GR = Germplasm Resources Laboratory, Beltsville, MD, H = Hernández, J = Johnson, L = Lott, M = Martinez, MFR = Forero-Romeo, MS = Sousa, N = Nee, R = Robbertse, S = Shilom, SM = Smith, T = Torres.

Zapoteca

Z. caracasana J-2494, MO; *Z. formosa* T-4262, T-4063, MO; *Z. lambertiana* S-3405, MO; *Z. media* D-53, MO; *Z. portoricensis* H-154, H-465, MO; *Z. tetragona* J-2246-80, J-1532-80, H-800, H-827, MO; *Z. sp. nov.* (1), T-4167, MO; *Z. sp. nov.* (2) T-3947, MO.

Calliandra

Macrophyllae: *C. angustifolia* 15051, COL; *C. carbonaria* 33509, COL, SM-6471, MO; *C. glaberrima* 55261, 117125, F-9325, COL; *C. mexicana* 6064, USF; *C. rekoii* MS-12545, MO; *C. seemannii* 1860709, F; *C. tergemina* 156125, COL; *C. aff. tergemina* L-1684, L-1686, H-166, H-768, MO.

Nitidae: *C. caeciliae* 1765611, F; *C. depauperata* MFR-607, COL; *C. eriophylla* H-191, MO; *C. glomerulata* 15864, COL; *C. haematocephala* 3817, USF; *C. humilis* D-1201, MO; *C. magdalenae* N-23070, H-674, MO; *C. matisiana* 25785, COL; *C. medellinensis* F-9415, COL; *C. pittieri* 66977, COL; *C. pubiflora* MFR-603, COL; *C. purdiei* F-9955, COL; *C. purpurea* F-9920, 05360, COL; *C. redacta* R-1168, MO; *C. reticulata* 1437, TEX; *C. rigida* 52044, COL; *C. rubescens* DS-11139, H-762, H-763, J-12273 MO, 1633009 F; *C. schultzei* F-9419, F-9861, F-9888, F-9889, F-9890, F-9897 COL; *C. selloi* MFR-538 COL; *C. tenuiflora* 133081, COL; *C. tolimensis* 14913, COL; *C. turbinata* 128406, COL; *C. sp. nov.* (1), H-867, MO.

Racemosae: *C. calothyrsus* GR s.n., MO; *C. grandiflora* H-113, T-4316, H-833, MO; *C. houstoniana* DN-5482, H-164, MO; *C. aff. houstoniana* M-6369, H-777, H-846, H-847, MO; *C. sp. nov.* (2), H-454, MO; *C. juzepezukii* H-400, MO; *C. parviflora* 1841137, F; *C. rusbyi* H-865, MO.

TABLE 2. Distribution of imino acids in leaves and seeds of *Zapoteca* species. TT (trans-trans), CC (cis-cis), TC (trans-cis), CT (cis-trans)-dihydroxypipelic acid; C4 (cis-4), T4 (trans-4), C5 (cis-5), T5 (trans-5)-hydroxypipelic acid; PIP—pipelic acid.

Species	Col. No.	TT	CC	TC	CT	C4	T4	C5	T5	PIP
<i>Z. caracasana</i>	J-2494	++					++	+		+
<i>Z. formosa</i>	T-4262	++					+	++		++
	T-4063	++					+	+		+
<i>Z. lambertiana</i>	S-3405	++					++	++		+++
<i>Z. media</i>	D-53	++					+	+		
<i>Z. mollis</i>	G-4164	++	++				+			+
<i>Z. portoricensis</i>	H-154	++				+		++		+
	H-465	++				+		++		+
<i>Z. tetragona</i>	J-2246-80	++					++	++		
	J-1532-80	++					++	++		
	H-800	++				+	++	++		+
	H-827	++				+	++	++		+
<i>Z. sp. nov. 1</i>	T-4167	++					+	+++		
<i>Z. sp. nov. 2</i>	T-3947	++					+	++		

+++ = >1 mg/g dry wt; ++ = 0.1–1 mg/g dry wt; + = <0.1 mg/g dry wt.

poteca, which includes those members of *Calliandra* belonging to Bentham's series *Laetevirentes*. All species of *Calliandra* that have been examined contain S-CEC as the major free seed amino acid (Table 1). This compound accounts for as much as 2.5% of the total seed weight. S-CEC has not been detected at all in members of *Zapoteca* (Table 1). The distribution of a second compound, S-CIC, which is found in much lower concentrations, follows the same pattern. Conversely, another nonprotein amino acid (S-2), as yet unidentified because of insufficient material but believed to be a sulphur compound, is the major free amino acid of *Zapoteca*. This compound is lacking or present only in trace amounts in *Calliandra* (Table 1).

The distributional data for the imino acids of *Zapoteca* are shown in Table 2. The presence of large amounts of trans-trans-4,5-dihydroxypipelic acid together with the monohydroxypipelic acids trans-4- and cis-5- is the common pattern. The dihydroxy isomer contains the same absolute configuration about the 4- and 5-carbons as the monohydroxy isomers with which it most frequently co-occurs. This *Zapoteca* pattern is one of several different combinations of mono and dihydroxy compounds that are seen in *Calliandra* species (Romeo, 1984 and unpubl. data). Of the other three dihydroxypipelic acid isomers that may characterize some sections of *Calliandra*, only the cis-cis isomer was detected in significant concentrations in one species of *Zapoteca*.

DISCUSSION

Nonprotein amino acids are useful as chemical systematic markers in legumes (see Bell, 1971, and Harborne & Turner, 1984 for reviews). Some taxa have been characterized by the accumulation of a single unusual amino acid. For example *Mucuna* species are distinguished by the accumulation of large amounts of L-3,4-dihydroxyphenylalanine (Bell & Janzen, 1971), and 5-hydroxy-L-tryptophan is found only in species of *Griffonia* (Bell et al., 1976). More often, however, it is an association of nonprotein amino acids that characterizes a taxon. Biochemical subgeneric groupings have been established for *Lathyrus* and *Vicia* (Bell, 1971), and *Acacia* (Seneviratne & Fowden, 1968; Evans et al., 1977). Recently, a reassessment of four major genera (*Derris*, *Tephrosia*, *Lonchocarpus*, and *Milletia*) of the Tephrosieae (Fabaceae) was suggested by studies of the distribution of basic nonprotein amino acids in this group (Evans et al., 1985).

The significance of the findings reported herein is difficult to evaluate for several reasons: 1) a limited number of species have been examined for sulphur compounds (8 of 18 taxa of *Zapoteca*, 39 of the ca. 200 species of *Calliandra*). Bentham's four major series are well represented in this group, however, and many species are represented by more than one sample (Table 1); 2) some chemical identities and the metabolic pathways of the secondary compounds are not yet known; 3) relationships within the large genus *Calliandra* are still unclear, and a suitable mor-

phological framework upon which to base chemical interpretation does not exist. Nonetheless, tentative judgements can be made. The sulphur amino acid patterns are distinctive enough that, on the basis of these data alone, one can assign species to one or the other group with a reasonable amount of certainty. When considered together with the palynological data (Guinet, 1965, 1969; Nevling & Elias, 1970; Niezgoda et al., 1983) and the morphological, cytological, ecological, and developmental findings (Hernández, 1986), these chemical data can be viewed as support for the segregation of the *Zapoteca* species from *Calliandra*.

The imino acid pattern of *Zapoteca*, in contrast, while generally consistent within the genus, is not a particularly distinctive one vis à vis *Calliandra*. Imino acid patterns observed in *Calliandra* consist primarily of the absence or presence of one or more dihydroxypipicolinic acid isomers in association with the monohydroxylated compounds that are the probable precursors of those isomers. All four possible dihydroxy compounds have discontinuous distributions within *Calliandra*. The usual trans-trans, trans-4, cis-5 pattern of *Zapoteca* is common in *Calliandra* and is found in several Latin American species. This sharing of rare imino compounds emphasizes the close phyletic relationship of *Zapoteca* to *Calliandra* just as the differing sulphur amino acids emphasize their distinctness. Since the distribution of these compounds is not known from other related genera (*Inga*, *Lysiloma*, *Pithecellobium*), it is not yet possible to speculate about the apomorphic or plesiomorphic nature of the chemical characters. As the metabolic relationships of the various compounds become elucidated, and as interspecific relationships within *Calliandra* itself become better understood with systematic revision, we can expect new phylogenetic insight into the relationship of *Zapoteca* to *Calliandra*.

LITERATURE CITED

- BELL, E. A. 1971. Comparative biochemistry of non-protein amino acids. Pp. 179–206 in J. B. Harborne, D. Boulter & B. L. Turner (editors), *Chemotaxonomy of the Leguminosae*. Academic Press, New York.
- & D. H. JANZEN. 1971. Medical and ecological considerations of L-DOPA and 5-HTP in seeds. *Nature* 229: 136–137.
- , L. E. FELLOWS & Y. M. QUERESHI. 1976. 5-hydroxy-L-tryptophan: taxonomic character and chemical defense in *Griffonia*. *Phytochemistry* 15: 823.
- BENTHAM, G. 1875. Revision of the suborder Mimosaeae. *Trans. Linn. Soc. London* 30: 335–664.
- BLEECKER, A. B. & J. T. ROMEO. 1981. 2,4-Trans-4,5-trans-4,5-dihydroxypipicolinic acid and cis-5-hydroxypipicolinic acid from leaves of *Calliandra angustifolia* and sap of *C. confusa*. *Phytochemistry* 20: 1845–1846.
- & ———. 1983. 2,4-Cis-4,5-cis-4,5-dihydroxypipicolinic acid—a naturally occurring imino acid from *Calliandra pittieri*. *Phytochemistry* 22: 1025–1026.
- EVANS, C. S., M. Y. QURESHI & E. A. BELL. 1977. Free amino acids in the seeds of *Acacia* species. *Phytochemistry* 16: 565–570.
- EVANS, S. V., L. E. FELLOWS & E. A. BELL. 1985. Distribution and systematic significance of basic nonprotein amino acids and amines in the Tephrosieae. *Biochem. Syst. Ecol.* 13: 271–302.
- FORERO, E. 1984. Revision of *Calliandra*: a multidisciplinary approach. *Bull. IGSM* 12: 14–15.
- GUINET, P. 1965. Etude des caractères du pollen dans le genre *Calliandra* (Mimosaceae). *Pollen et Spores* 7: 157–173.
- . 1969. Les Mimosacées. Etude de palynologie fondamentale, corrélations évolution. *Trav. Sect. Sc. Tech. Inst. Fr. Pondichery* 9: 1–293.
- HARBORNE, J. B. & B. L. TURNER. 1984. *Plant Chemotaxonomy*. Academic Press, New York.
- HERNÁNDEZ, H. M. 1986. *Zapoteca*: a new genus of Neotropical Mimosoideae. *Ann. Missouri Bot. Gard.* 73: 755–763.
- KRAUSS, G. J. & H. REINBOTHE. 1970. Die Aminosäuren der Gattung *Albizzia*. *Biochem. Physiol. Pflanzen* Bd. 161: 243–265.
- MARLIER, M., G. A. DARDENNE & J. CASIMIR. 1972. 4,5-Dihydroxy-L-pipécolique a partir de *Calliandra haematocéphala*. *Phytochemistry* 11: 2597–2599.
- , ———, & ———. 1979. 2S,4R-Carboxy-2-acétylamino-pipéridine dans les feuilles de *Calliandra haematocéphala*. *Phytochemistry* 18: 479–481.
- NEVLING, L. I. & T. ELIAS. 1970. *Calliandra*, pollinia and systematic implications. *Amer. J. Bot.*, Suppl. 59: 753.
- NIEZGODA, C. J., S. M. FEUER & L. I. NEVLING. 1983. Pollen ultrastructure of the tribe Ingeae (Mimosoideae). *Amer. J. Bot.* 70: 650–667.
- ROMEO, J. T. 1984. Preliminary chemotaxonomic investigations of Colombia *Calliandra* species based on nonprotein imino acids. *Bull. IGSM* 12: 19–23.
- , L. A. SWAIN & A. B. BLEECKER. 1983. Cis-4-hydroxypipicolinic acid and 2,4-cis-4,5-trans-4,5-dihydroxypipicolinic acid from *Calliandra*. *Phytochemistry* 22: 1615–1617.
- SWAIN, L. A. & J. T. ROMEO. 1986. Persistence of the nonprotein seed amino acid S-(β -carboxyethyl)-cysteine in young leaves of *Calliandra rubescens*: ecological implications. *J. Chem. Ecol.* 12: 2089–2096.
- SENEVIRATNE, A. S. & L. FOWDEN. 1968. The amino acids of the genus *Acacia*. *Phytochemistry* 7: 1039–1045.